



Anti ulcer activity of ethanolic extract of fruit of *Trapa bispinosa* Roxb in animals

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Abstract

*The antiulcer activity of the fruits of *Trapa bispinosa* (Trapaceae) was studied on wistar rats. The antiulcer activity of 50% ethanolic extract at two dose levels was evaluated by using pyloric ligation and aspirin plus pyloric ligation models. The tests extract revealed significant antiulcer activity, which might be due to increase in total carbohydrate content and alter state of mucosal barrier of the stomach. The results indicate that the ethanolic extract of fruits of *Trapa bispinosa* is endowed with potential antiulcer activity.*

Key words: Pylorus ligation, Fucose, Siali acid, Total carbohydrate, Water chestnut

INTRODUCTION

The selection of scientific and systemic approach for the biological evaluation of plant world on the basis of their use in the traditional system of medicine forms the basis for an ideal approach in the development of new drugs from plants. Peptic ulcer is known to develop either due to increased gastric acid and pepsin secretion or a reduced mucosal defense or by a combination of these two.

Trapa bispinosa Roxb (*Trapaceae*), commonly known as Water chestnut, is an aquatic herb occurring throughout the greater parts of India in lakes, tanks and ponds [1]. The fresh tender kernels are sweet, delicious and farinaceous and the flavour resembles that of chestnut. The fruits are reported to be used in the indigenous system of medicine for treating a variety of ailment like leprosy, burning sensation, fatigue, inflammation, biliousness, strangury and fractures[2,3]. In Unani system of medicine, the fruits are said to be used as aphrodisiac, antipyretic and useful in biliousness, bronchitis, pain and sore throat[3]. Rahman *et al* [4] reported antibacterial and

cytotoxic activity of the fruits. The tribes of Orissa use the fresh fruits orally for the treatment of gastrointestinal disorders, besides its use as an edible food. Reports on the biological activities of the fruits are very limited. Hence, an attempt was made to investigate the anti ulcer activity of the fruits in standard experimental animal models.

MATERIALS AND METHODS

Plant material

The fruits of *trapa bispinosa* were collected from a local pond in Bhubaneswar and authenticated by the taxonomists of Department of Botany, Utkal University, Bhubaneswar. The fresh fruits, thus collected in bulk, were washed, shade dried and broken down into pieces and then milled into coarse power by a mechanical grinder. The powder was passed through sieve no. 40 and extracted with petroleum ether (40-60⁰C) in a soxhlet extractor. The defatted material was subsequently extracted with 50% ethanol. The liquid extract was concentrated under vacuum and dried in a desiccator. The dried extract was suspended in 0.5% w/v sodium carboxymethyl cellulose in distilled water (vehicle) and used for study.

Animals study

Adult albino Wistar rats (150-200g) and Swiss albino mice (25-30g) of either sex, (M/S Ghose Enterprises, Kolkata) used in the experiment were allowed to acclimatize to the laboratory conditions for 7 days in acrylic cages prior to commencement of the experiment with 12 hr day and night schedule at a temperature of 26±4⁰C. The animals were maintained with standard pellet diet and water *ad libitum*.

Acute Oral Toxicity Study

Approximate lethal dose (ALD₅₀) was calculated according to Smith[5] on the selected mice, which were randomly divided and housed in four groups of 4 animals each in laboratory conditions. The animals were fasted overnight but had free access to water. The test extract was administered separately at dose level of 1000, 2000, 4000 and 8000 mg/kg through oral route as single dose. The animals were subjected to primary screening studies at 0.5, 1, 2 and 4hr and for mortality, if any, up to 14 days in the observation chambers.

Pyloric ligated ulceration

This test was performed as suggested by *Shay et al.*[6]. The rats were divided randomly into four groups of six animals each. Each group of the animals received one of the following test samples through oral route: 0.5% w/v sodium carboxymethyl cellulose in distilled water (2 ml/kg); ranitidine (25 mg/kg), test extract (250 and 500 mg/kg) respectively, twice daily for two days. One hour after the last treatment, pylorus ligation was done under ether anesthesia. The animals were then returned to the observation chamber. The animals were deprived of both food and water during the postoperative period. After 4 hrs, the animals were sacrificed by ether overdose, the abdomen of each animal was opened and the stomach was isolated after suturing the lower esophageal end. The gastric juice was collected by giving a small cut to the pyloric region just above the knot in a measuring cylinder and stomach was opened along the greater curvature. The mucosal layer was washed with 1ml-distilled water and the washings were added to the gastric secretions. The gastric contents were centrifuged at 2000 rpm for 10 minutes. The volume

and pH was recorded and subjected to biochemical estimations like free acidity, Total acidity, total protein, Hexosamine, Pepsin, Fucose and Sialic acid.

Each stomach was then examined carefully for characterizing severity of ulcer. The ulcer was graded as suggested by Kuchandy *et al*[7] and Kulkarni[8]. 0= Normal coloured stomach; 0.5= Red colouration; 1= Spot ulcer; 1.5= Hemorrhagic streaks; 2= Ulcers ≥ 3 but ≤ 5 ; 3 = Ulcers > 5 . The mean ulcer score of each animal was expressed as ulcer index. The results are presented in table no. 1 and 2

Estimation of free acidity and Total acidity

The supernatant fluid (1ml) of the gastric juice was diluted with 9ml of distilled water and then titrated against 0.01N sodium hydroxide solution using Toffer's reagent till the solution turns orange in colour. The volume of sodium hydroxide required corresponds to free acidity. The solution was further titrated till the solution regained pink colour. The volume of sodium hydroxide required corresponded to the total Acidity[9].

Estimation of Total Proteins

The dissolved protein in gastric juice was estimated in the alcoholic precipitate obtained by adding 90% alcohol with gastric juice 9:1 ratio respectively. Then 0.1 ml of alcoholic precipitate of gastric juice was dissolved in 1ml of 0.1N NaOH and from this 0.05 ml was taken in another test tube. To this 4 ml of alkaline mixture was added and kept for 10minutes. Then 0.4 ml of phenol reagent was added and again 10minutes was allowed for colour development. Reading was taken against blank prepared with distilled water at 610 nm, in Hitachi 15-20 spectrophotometer. The protein content was calculated in terms of $\mu\text{g/ml}$ of gastric juice[10].

Estimation of Total Carbohydrates

The dissolved mucosubstances in gastric juice were estimated in the alcoholic precipitate obtained by adding 1 ml of gastric juice to 9 ml of 90% alcohol, the mixture was kept for 10 minutes, and the supernatant was discarded. The precipitate separated was dissolved in 0.5 ml of 0.1N sodium hydroxide. To this 1.8ml of 6N, HCl was added. This mixture was hydrolysed in the boiling water bath for 2 hrs. The hydrolysate was neutralized by 5N sodium hydroxide using phenolphthalin as indicator and the volume was made up to 4.5 ml with distilled water and used for the estimation of total hexoses, hexosamine and fucose as described below[11].

Estimation of Total Hexoses

To 0.4 ml of hydrolysate, 3.4 ml of orcinol reagent was added. The mixture was then heated in the boiling water bath for 15 min. this was then cooled under running tap water and intensity of the colour was read in Hitachi 15-20 spectrophotometer at 540 nm against the blank by using distilled water instead of hydrolysate. Total hexoses content was determined from the standard curve of D (+)-galactose-mannose and has been expressed in $\mu\text{g/ml}$ of gastric juice[12].

Estimation of Hexosamine

0.5 ml of the hydrolysate fraction was taken. To this 0.5 ml of acetylacetone reagent was added. The mixture was heated in boiling water bath for 20 minutes. Then cooled under running tap water. 1.5 ml of 90% alcohol was then added followed by an addition of 0.5 ml of Ehrlich's reagent. The reaction was allowed for 30 minutes. The colour intensity was measured in 15-20

spectrophotometers at 530 nm against the blank prepared by using distilled water instead of hydrolysate. Hexosamine content of the sample was determined from the standard curve of D (+)-galactose-mannose and concentration has been expressed in $\mu\text{g/ml}$ of gastric juice[13].

Estimation of Fucose

In this method, three test tubes were taken. In one tube 0.4ml of distilled water was taken to serve as control and in each of the other two tubes 0.4 ml of hydrolysates were taken. To all three tubes 1.8 ml of sulphuric acid and water in the ratio of 6:1 was added by keeping the tubes in ice-cold water bath to prevent breakage due to strong exothermic reaction. This mixture was then heated in boiling water bath for exactly 30minutes. The tubes were then taken out and cooled. In the blank and one of the hydrolysate containing tubes (unknown) 0.1ml of cysteine reagent was added, while cysteine reagent was added to the last test tube containing the hydrolysate (unknown), it was then allowed for 90mm to complete the reaction. The reading was taken in Hitachi 15-20 spectrophotometer at 396 and 430 nm setting the zero with the distilled water. The optical density for the fucose in the hydrolysate was calculated from the differences in the reading obtained at 396 and 430 nm and subtracting the values without cysteine. This was read against standard curve prepared with D (+)-fucose. The fucose content was expressed in terms of $\mu\text{g/ml}$ of gastric juice[14].

Estimation of Pepsin

For each determination four tubes were placed and numbered as 1-4 in which 1 and 2 containing 5 ml of substrate 3 and 4 containing 10 ml of trichloroacetic acid. The gastric juice was mixed with an equal volume of HCl at pH 2.1 warmed to 37°C and 1ml of mixture was added to each tubes 1 and 4. After 25 min. incubation, 1+3 gives test and 2+4 gives blank. Filtered the content of the test tubes and kept for 30min. 2 ml of the filtrate was pipetted into 10 ml of sodium hydroxide and mixed by gentle rotation. After 30 minutes the intensity of colour was measured at 680 nm in Hitachi 15-20 spectrophotometer. The difference between test and blank gives a measure of peptic activity[15].

Estimation of Sialic acid

To 0.5 ml of the hydrolysate in 0.1N sulphuric acid, 0.2 ml of sodium periodate was added and mixed thoroughly by shaking. A time of 20minutes was allowed to elapse before addition of 1 ml of sodium arsenite solution mixture. The developed brown colour was disappeared by shaking. Then 3 ml of thiobarbituric acid was added and while shaking 4.5ml of cyclohexanone was added, till the entire colour was taken up by the cyclohexanone supernatant. The resulting mixture was centrifuged to get a clear pink layer of cyclohexanone. The supernatant was pipetted out and intensity of colour was measured in Hitachi 15-20 spectrophotometer at 550 nm. The sialic content of the sample was determined from the standard curve of sialic acid and has been expressed in $\mu\text{g/ml}$ of gastric juice[16].

Aspirin induced ulceration

The selected animals (rats) were divided randomly into four groups of six animals each. Each group of animals received the test samples twice daily for two days for both dose levels (250 and 500mg/kg) and the standard drug, ranitidine was administered once daily orally at a dose of 25mg/kg b.wt. for two days prior to and one hour before administration of aspirin. On second day (37^{th} hour) aspirin was administered at a dose of 200 mg/kg orally as a suspension prepared

in 0.5% w/v CMC with distilled water for all groups, one hour prior to pyloric ligation. The animals were deprived of food and water during the post operative period. Ranitidine (25 mg/kg) was used as reference standard. After 30 minute, each animal was administered 200 mg/kg aspirin through oral route. After 1hr, pylorus ligation was made as per the procedure[17]. The animals were killed after 4hr; the stomachs were opened along the greater curvature and carefully observed for severity of ulceration as described earlier and subjected to analysis for free acidity, Total acidity, Total protein, Hexosamine, Pepsin, Fucose and Sialic acid .The results are depicted in (Table-3 and 4)

Statistical Analysis

The results were analyzed statistically using Student's t-test where applicable. The level of significance for all determinations was fixed at $p < 0.01$.

RESULTS AND DISCUSSION

The results of the study reveal that the 50% ethanolic extract of the fruits of *trapa bispinosa* possess significant antiulcer activity. In both pyloric ligated ulceration (Shay rat model) and aspirin induced ulceration, the test extract in both the dose levels was found to reduce ulcer index to a significant extent in a dose dependent manner. In pylorus-ligated model, free acidity decreases with no significant change in the gastric volume, pH, total acidity and pepsin in both dose levels of the test extract. The presence of dissolved mucous substances in the gastric juice seems to be a reliable index of an effective mucosal barrier[18]. The protein content of the gastric juice suggests the mechanism of leakage of plasma protein into the gastric juice[19]. The test extract at both the dose levels produced a significant decrease in protein content and an increase in the carbohydrates content in a dose dependent manner. In pylorus ligated model, the ulcer is developed due to increased metabolism of carbohydrates and increased synthesis of nucleic acid and also exhaustion of carbohydrates and other compensatory mechanisms[20]. The increased carbohydrate content by the test extract may presumed to be responsible for altering mucous secretion which in turn alter status of mucosal barrier.

In aspirin plus pylorus ligated model the initial damage by aspirin, aggravates the damage further with increase acid secretion and decreased mucous production with increase pepsin activity. The pretreatment with 50% ethnolic extract may have role in the suppression of severity of ulcer due to presence of carbohydrates and other components present in the extract.

Table 1: Effect of fruits of *Trapa bispinosa* on pyloric ligated ulcer in rats

Treatment	Dose	Gastric volume (ml)	Ulcer Index	pH	Free acidity meq/l	Total acidity meq/l	Pepsin µg/ml
Solvent	2ml/kg	8.6±0.28	2.7±0.09	2.21±0.22	6.56±0.07	7.77±0.25	18.85±3.11
Ranitidine	25mg/kg	4.5±0.29**	0.49±0.08**	4.18±0.1**	3.43±0.08**	5.4±0.14**	6.35±0.34**
Extract	250mg/kg	6.3±0.82	1.28±0.09**	2.22±0.35	5.95±0.09*	7.5±0.12	13.67±0.29
	500mg/kg	7.25±0.72	0.58±0.07**	2.78±0.25	4.64±0.29*	7.45±0.08	10.67±0.88

Values expressed as Mean ± SEM from six observations. * $p < 0.01$, ** $p < 0.001$ when compared with solvent control

Table 2: Effect of *Trapa bispinosa* Fruits on biochemical parameters in pylorus ligated ulcer in rats

Treatment	Dose	Protein µg/ml	Total Hexose µg/ml	Hexosamine µg/ml	Fucose µg/ml	Sialic acid µg/ml	Total carbohydrate µg/ml	TC:P
Solvent	2mi/kg	757.5±4.25	140±1.15	292.8±2.14	71±1.48	30.38±2.18	534.18	0.71
Ranitidine	25mg/kg	294±3.28**	275.8±2.58**	378.2±3.21**	155.4±3.29	72.38±2.34**	881.78	3
Extract	250mg/kg	598±5.29**	175±3.29**	94.2±4.35**	61.4±2.28*	49±2.35**	379.60	0.63
	500mg/kg	325±4.8**	248.3±4.82**	182.7±3.7**	90.8±3.18**	68.0±2.43**	589.80	1.81

Values expressed as Mean±SEM from six observations. * $p < 0.01$, ** $p < 0.001$ when compared with solvent control.

Table 3: Effect of fruits of *Trapa bispinosa* on aspirin plus pyloric ligated ulcer in rats

Treatment	Dose	Gastric volume ml	Ulcer index	pH	Free acidity meq/l	Total acidity meq/l	Pepsin µg/ml
Solvent	2ml/kg	8.89±0.62	2.68±0.06	2.39±0.08	8.24±0.72	9.05±0.39	21.88±1.12
Ranitidine	25mg/kg	5.18±0.51**	0.44±0.09**	4.15±0.21**	4.08±0.46**	6.17±0.23**	5.32±0.36**
Extract	250mg/kg	8.25±0.55	2.24±0.1*	2.25±0.09	9.59±0.21	10.25±0.28	16.28±1.19
	500mg/kg	7.15±0.59	0.54±0.07**	2.59±0.08	9.12±0.39	10.8±0.48	10.58±2.46*

Values expressed as Mean±SEM from six observations. * $p < 0.01$, ** $p < 0.001$ when compared with solvent control.

Table 4: Effect of *Trapa bispinosa* Fruits on biochemical parameters in aspirine plus pylorus ligated ulcer on rats.

Treatment	Dose	Protein	Total Hexose µg/ml	Hexosamine µg/ml	Fucose µg/ml	Sialic acid µg/ml	Total carbohy- drate	TC:P
Solvent	2ml/kg	818.61±3.59	188.42±3.09	248.28±4.34	94.76±3.28	31.69±4.38	563.15	0.69
Ranitidine	25mg/kg	279.2±4.58**	282.5±4.58**	432.6±3.48	235.3±3.33**	76.2±4.39**	1026.6	3.68
Extract	250mg/kg	529.3±7.29**	196.6±3.12	272.9±5.38*	111.4±2.84*	55.33±4.54*	636.23	1.20
	500mg/kg	349.6±6.88**	243.3±4.42**	314.3±6.8**	198.6±4.05**	69.4±3.68*	815.6	2.33

Values expressed as Mean±SEM from six observations. * $p < 0.01$, ** $p < 0.001$ when compared with solvent control.

CONCLUSION

The 50% ethanolic extract of the water chestnut endowed with antiulcer potential possible due to presence of carbohydrate in the test extract. The test extract has significant antiulcer potential in a dose dependent manner when compared with solvent (saline) treated animals.

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